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Fig. 1a

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Fig. 1b
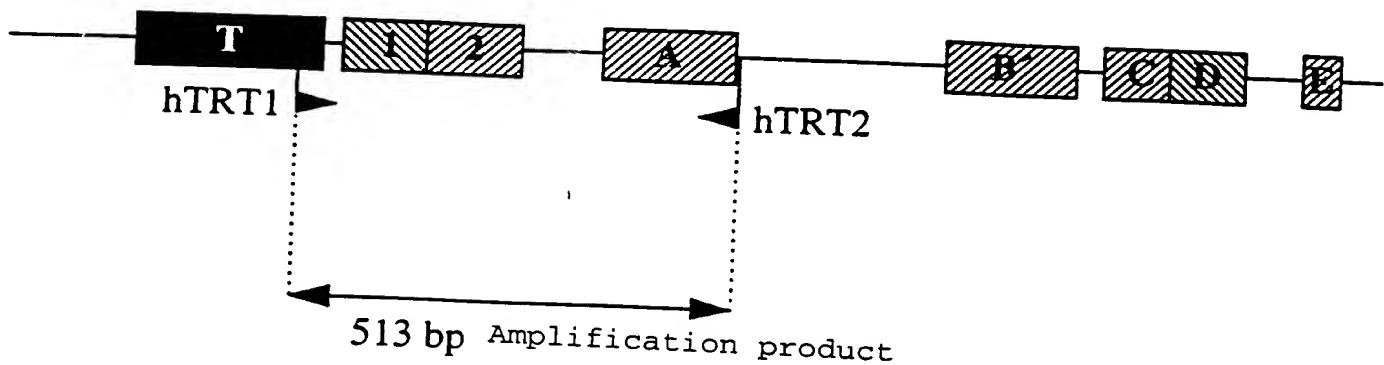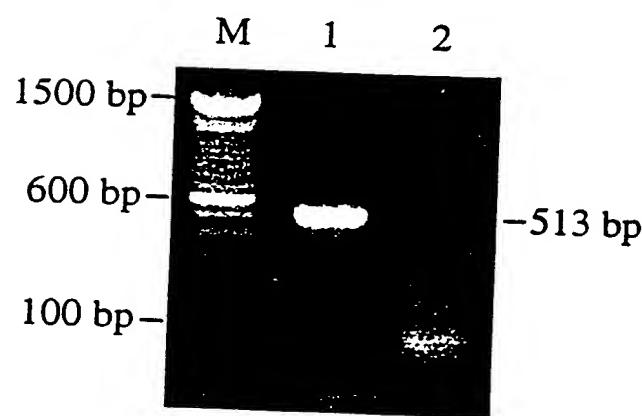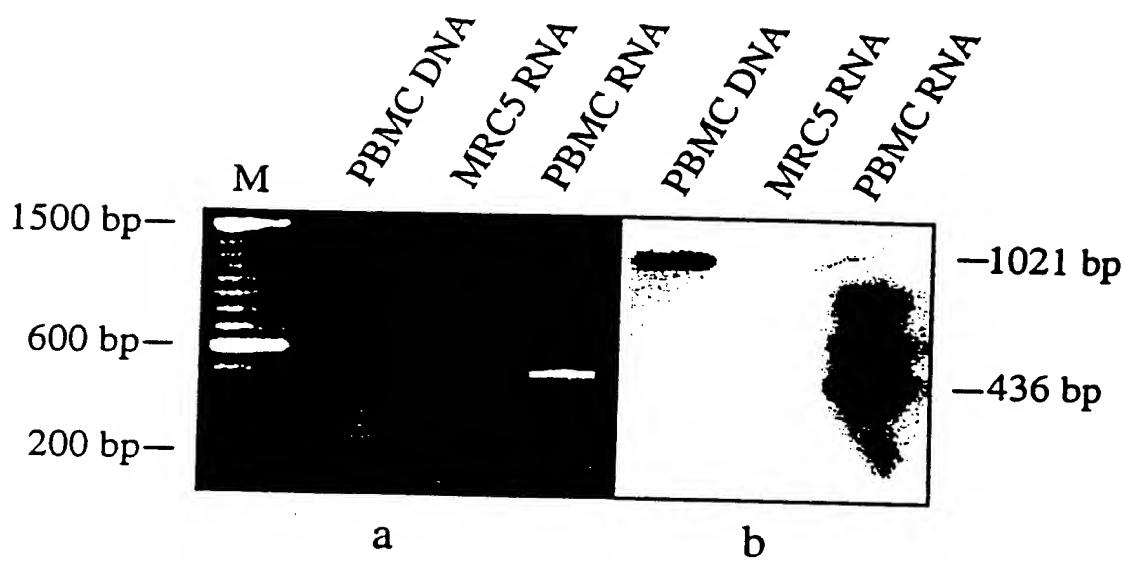
A

513 bp Amplification product

B

Fig. 2

Fig. 3

A)

B)
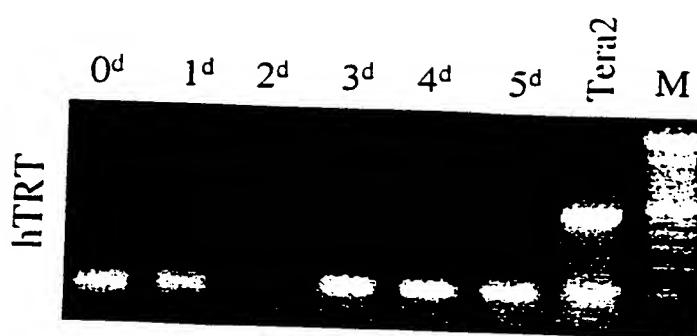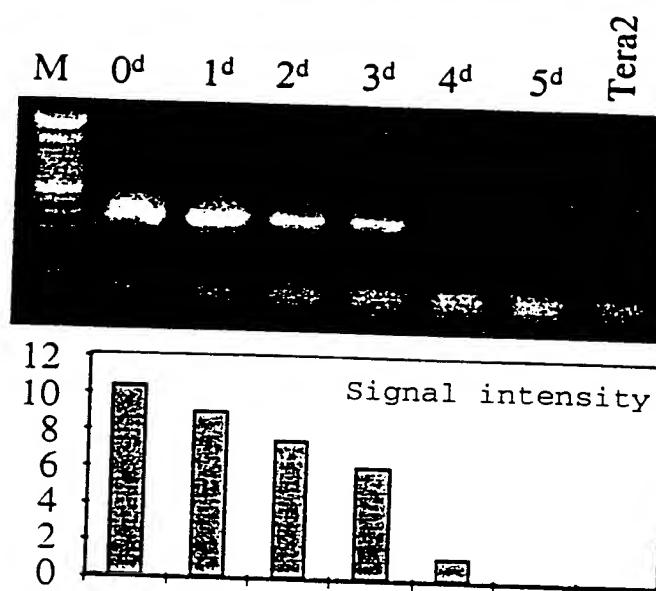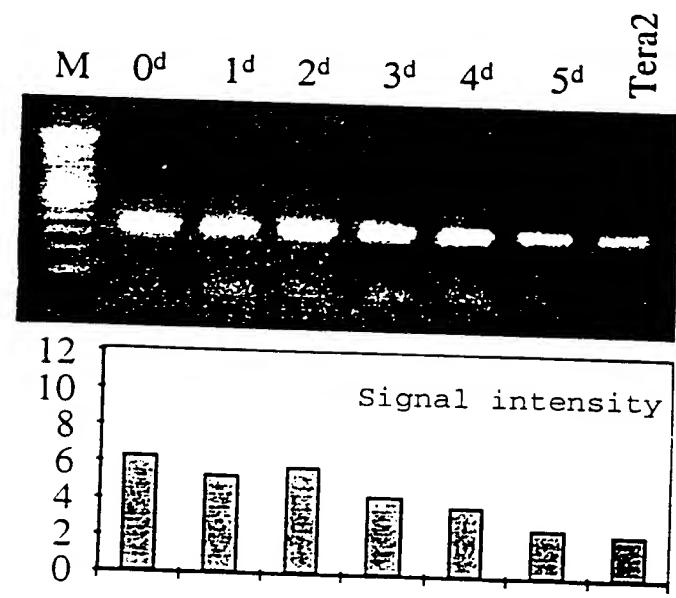
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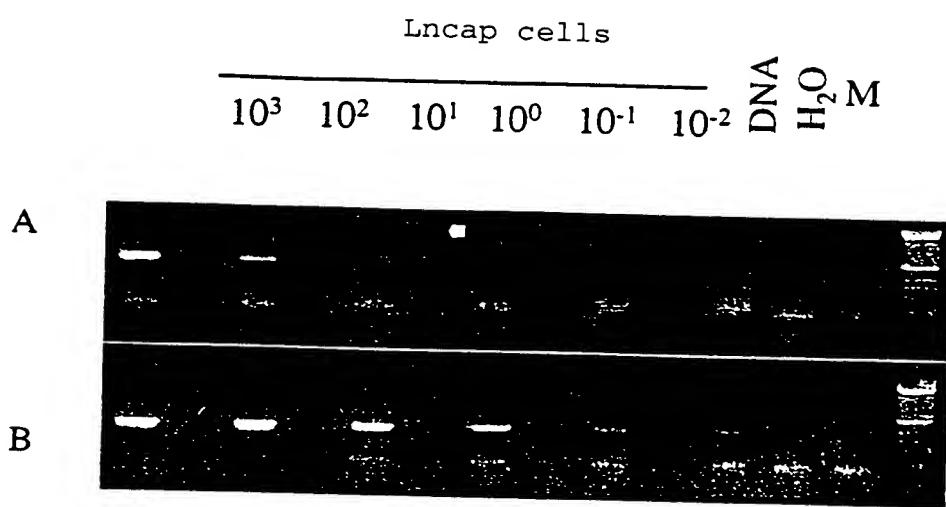
Fig. 5

Catalytic sububit of human telomerase

Name	Sequence (5`-3)
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hTRT o	CGTTCTGGCTCCCACGACGTAGTC

T-cell receptor:

Name	Sequence (5`-3)
TCR 1	GAGGTCGCTGTGTTTGAGCCATCAGAAG
TCR 2	GATCTCATAGAGGATGGTGGCAGACAG

β-actin:

Name	Sequence (5`-3)
Act 1	GATGATGATATCGCCGCGCTCGTC
Act 2	CTCAAACATGATCTGGGTCATCTTC

β-globin:

Name	Sequence (5`-3)
Glob 1	ACCCAGAGGTTCTTTGAGTC
Glob 2	TCTGATAGGCAGCCTGCACT

Fig. 6

Whole blood/Ficoll-isolated PBMC

RNA isolation using the 'whole blood protocol or standard methods such as phenol/chloroform or silica gel column

total RNA

Amount of RNA corresponding to a certain vol. (e.g. 1 ml) of whole blood

buffer : 100 mM Tris/Cl,
pH 8.3
50 mM KCl
5 mM $MgCl_2$
1 mM dNTP- mix
2.5 µM random hexamer
4U Rnase-free DNase
40U RNase inhibitor
in a volume of 36 µl

DNase- digest

30'/37°C, 10'/75°, 10"/90°C
→ immediately on ice

sample (18 µl)   negative control (18 µl)

+ 50U MuLV RT
+ 40U Rnase inhibitor

+ 4 µl DEPC water

reverse traskriptase reaction

30'/42°C, 5'/99°

cDNA   (cDNA)

buffer : 100 mM Tris/Cl,
pH 8.3
50 mM KCl
2 mM $MgCl_2$
200 µM dNTP- mix
2.5U AmpliTaq DNA
polymerase
300 µM per primer
in a volume of 25 µl

PCR- reaction 1)

analysis/quantification

1) PCR- conditions :

15"/97°C(15"/97°C,30"/70°C,-0.5°C/cycle,30"/72°C)x10
(15"/94°C,30"/65°C,-0.5°C/cycle,30"/72°C)x20
(15"/94°C,30"/50°C,30"(ext.15"/cycle)/72°C)x10
7'/72°C

Fig. 7

Fig. 8

B)

Tumor cells for each 4 ml of blood	Tumor cells for each PCR batch
0	0
16	4
32	8
64	16
128	32
256	64

without blood

| 256 | 64 |
| 10^4 | 2500 |

Actin
CK20
hTR
hTRT
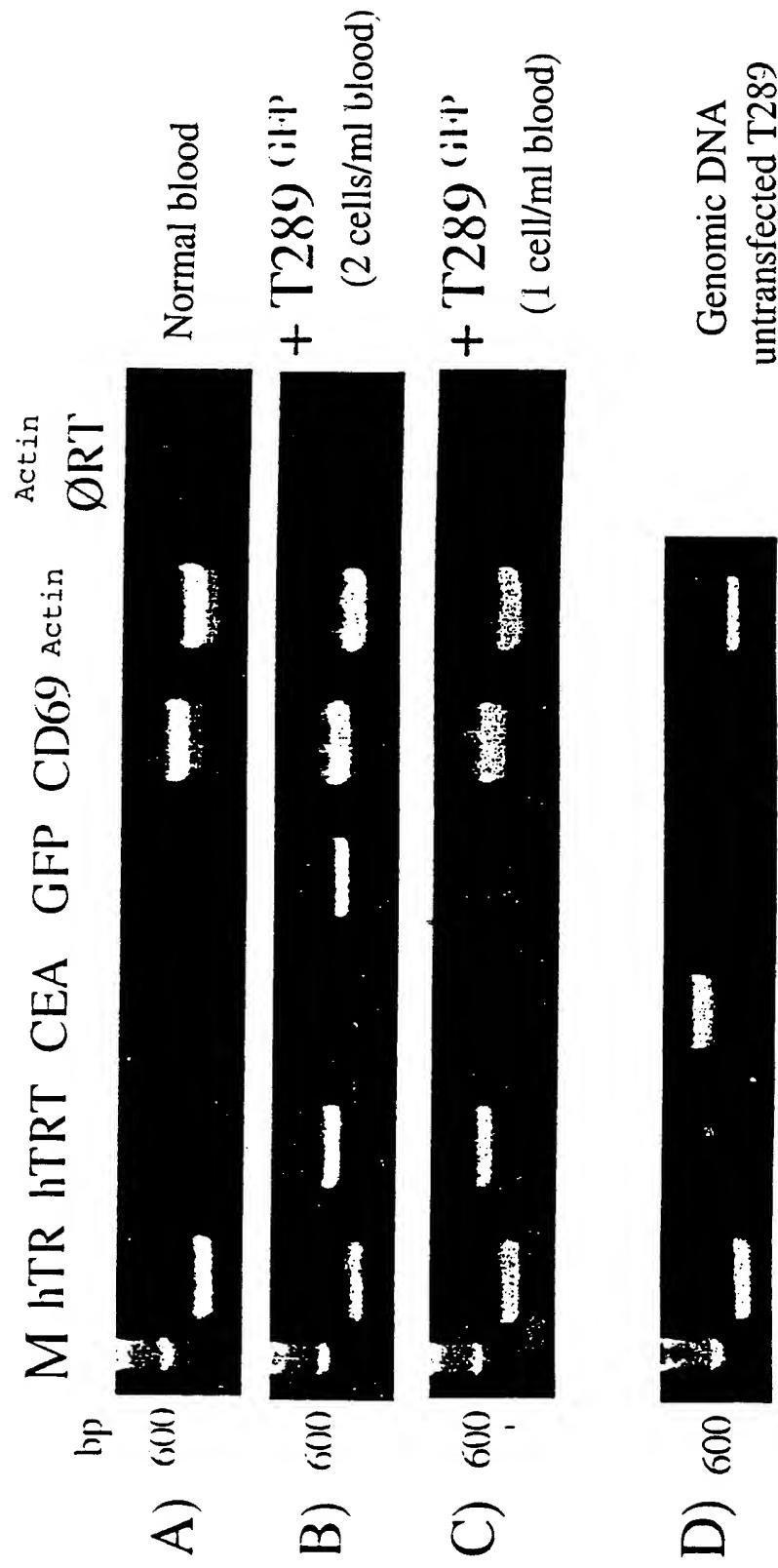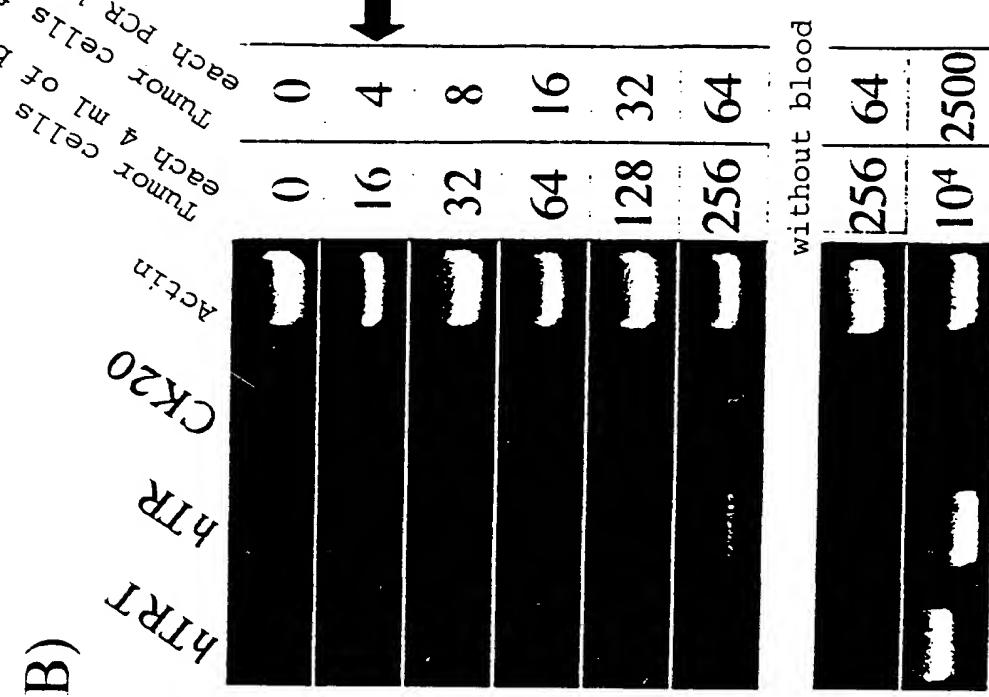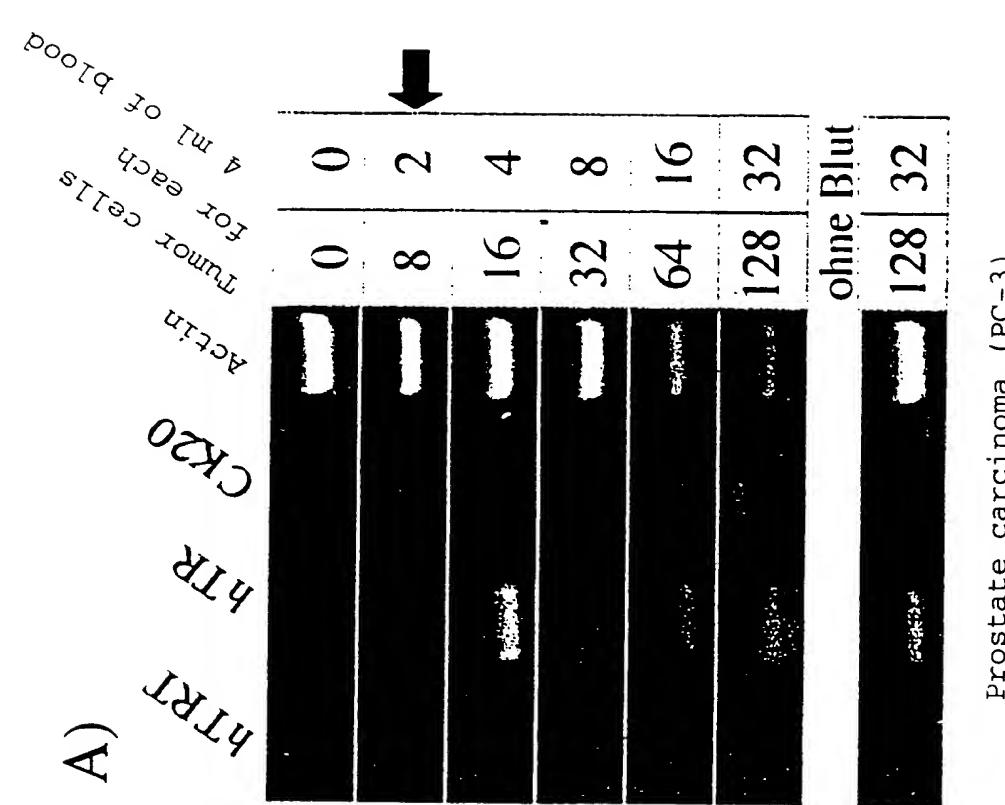
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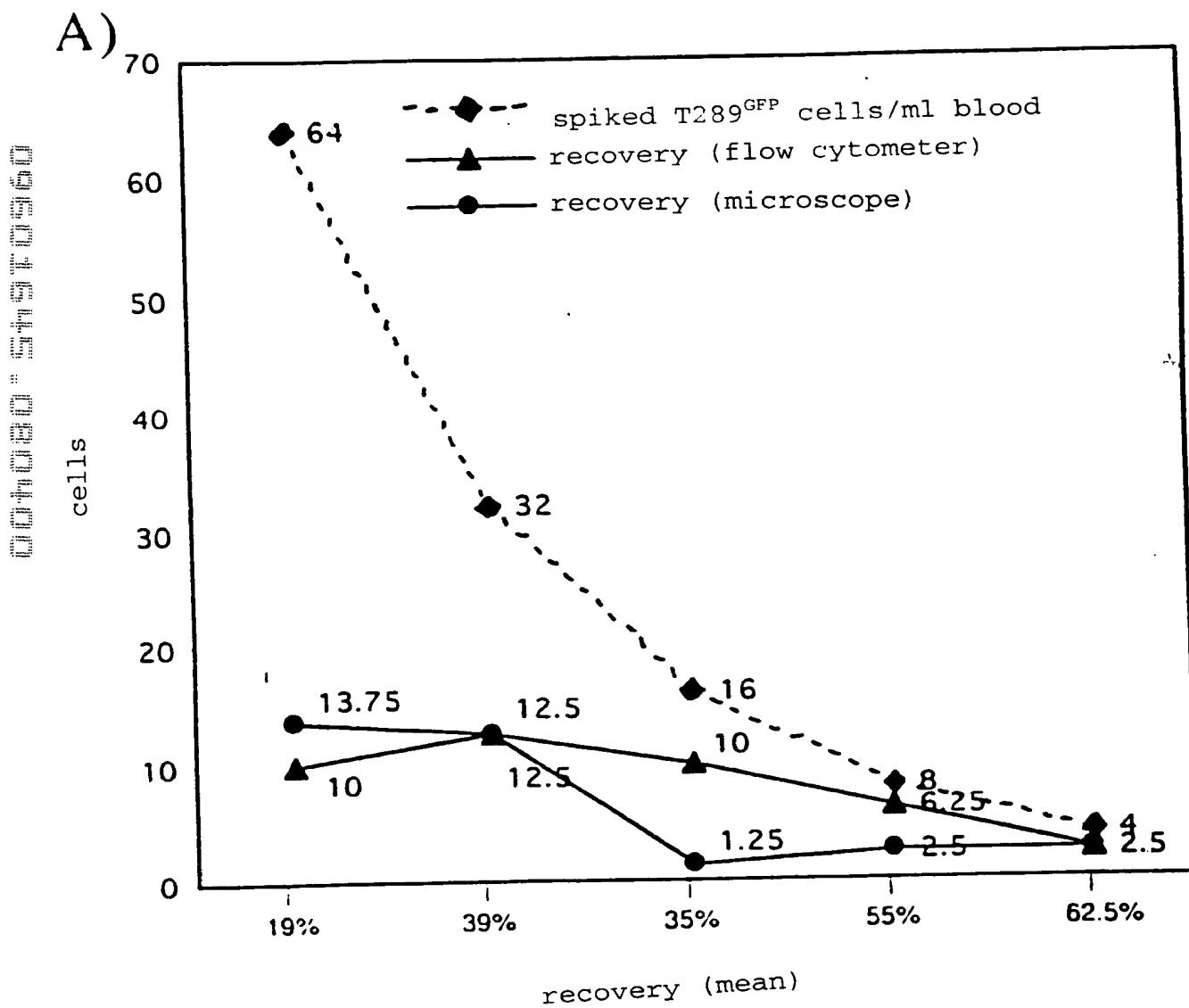
A)

Tumor cells for each 4 ml of blood	Tumor cells for each 4 ml of blood
0	0
8	2
16	4
32	8
64	16
128	32

ohne Blut

| 128 | 32 |

Actin
CK20
hTR
hTRT

Prostate carcinoma (PC-3)

Fig. 9

- 59a -

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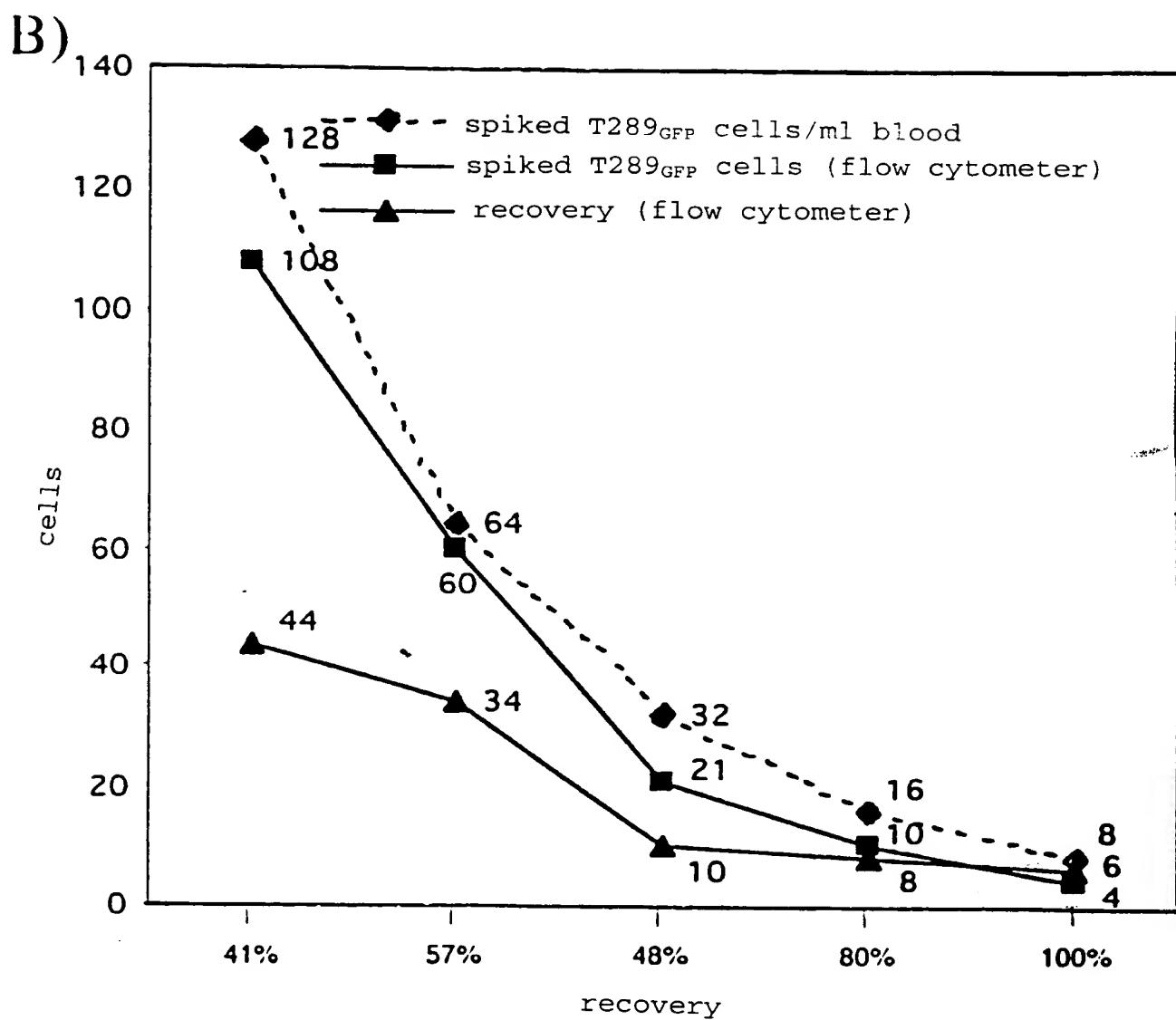
Fig. 10

09/601645

Fig. 10

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